Statistical report

This document sets out our statistical model's assumptions, explains its implementation and presents the results.

Background

The aim of this analysis is to describe timecourse data about the density of CHO cell cultures that were given treatments that induce cell death through a process called apoptosis. Some cultures were given genetic interventions that aim to make them apoptosis-resistant, either by reducing the rate at which they die or by extending the period before they start to die. We would like to know which interventions have the most effect, and in what way.

Methods

Assumptions about the target system

We assume that in this scenario the cells exist in four states:

- R replicative, growing at a rate of $\mu R(t)$, where t is the current time and $R(t)$ is the current density of replicative cells
- Q_a growth arrest, transferring from normal at a rate of $k_q R(t)$
- Q_c death committed, transferring from growth arrest at a rate of $k_q R(t-\tau)$, where $tau > 0$ represents the delay between growth arrest and death commitment.
- dead, transferring from death committed at a rate of $k_d Q_c(t)$, where $Q_c(t)$ is the density of death-committed cells at time t.

These assumptions define a system of ordinary differential equations (specifically delay differential equations) that can be solved analytically, so that the density at a given time can be found as a function of the parameters μ, τ k_q, k_d and the initial density of replicative cells $R0$.

We have measurements of the total cell volume, i.e. $R(t) + Q_a(t) + Q_c(t)$ at several time points, for cell cultures with the following structure:

- 9 genetic designs, comprising 7 genetic interventions and two control designs.
- Between 1 and 4 clones implementing each design
- Two technical replicates for each clone.

Replicates of the same clone are biologically identical, though we expect some variation in measurements due to the experimental conditions. Clones with the same design are expected to be similar, with some degree of clonal variation that

may differ depending on the design. There is no prior information distinguishing the designs from each other, or distinguishing clones with the same design.

Given these assumptions a multi-level Bayesian statistical model is appropriate.

Measurement model

We used the following measurement model:

$$y \sim log\, student\, t(\log(\hat{y}(t, R0, \mu, \tau, k_q, k_d)), \sigma)$$

where

- $R0$, τ, k_q and k_d are vectors of clone-level parameters.
- μ is a model parameter representing the pre-treatment growth rate, which we assume is the same for all replicates.
- σ is an unknown log-scale error standard deviation.
- t is a vector of known measurement times (one per measurement).
- $\hat{y}$ is a function mapping parameter configurations to densities, under the delay differential equation assumptions laid out above.

We believe that the measurement error will be proportional to the true viable cell density for most measurements, motivating the use of the lognormal generalised linear model. However, we hypothesise that for cell densities below 0.3 this will not be the case, as for these measurements the error is dominated by factors that do not depend on the true cell density, such as impurities in the apparatus.

To allow the model to incorporate this postulated effect we use the following distributional model:

$$\sigma = exp(a_\sigma + b_\sigma * \min(0, \ln(\hat{y} - 0.3)))$$

In this equation the parameter b_σ represents the degree to which the log-scale measurement error increases or decreases as the true value gets lower than 0.3.

Code to generate figure 1:

```
import numpy as np
from matplotlib import pyplot as plt

y = np.linspace(0.04, 0.4, 20)
bs = [-0, -0.05, -0.1, -0.15, -0.2]
diff = np.array([np.log(yi/0.3) if yi < 0.3 else 0 for yi in y])
for b in bs:
    plt.plot(y, 0.2 + b * diff, label=str(b))
plt.legend(title="$b_\sigma$")
plt.xlabel("True density")
```

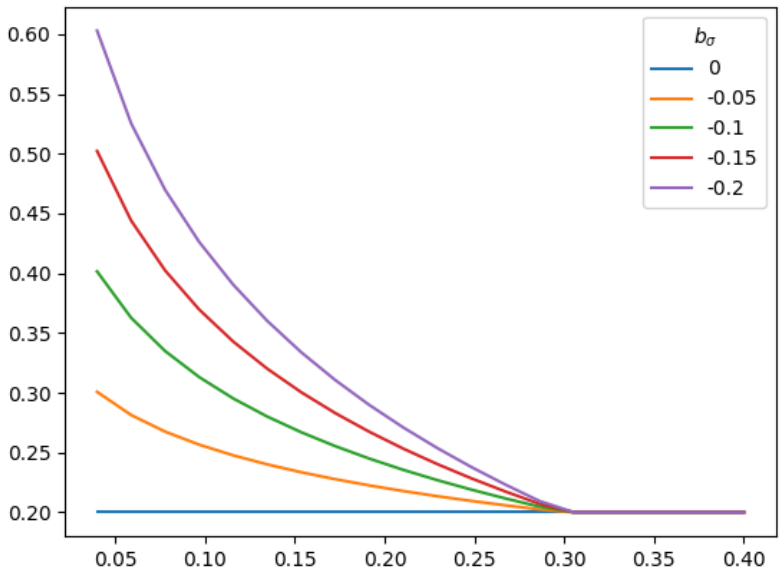

Figure 1: True density vs ln scale measurement standard deviation for a range of b_σ values

```
plt.xlabel("ln scale standard deviation")
plt.savefig("results/y_vs_log_sd.png")
```

Design level parameters

The clone-level vectors τ_r and k_d are treated as determined by other parameters as follows:

$$\ln(\tau) = \tau const + d_\tau * X + c_{tau}$$
$$\ln(k_d) = dconst + d_d * X + c_d$$

In these equations

- $qconst$, $\tau const$ and $dconst$ are single unknown numbers representing the (log scale) mean parameter values with no interventions
- X is a matrix indicating which clones have which interventions
- d_τ and d_d are vectors of unknown intervention effects
- c_τ and c_d are vectors of unknown clone effects, representing random clonal variation.

The priors for

$$d_\tau \sim N(0, 0.3)$$
$$d_d \sim N(0, 0.3)$$

To investigate whether the genetic interventions measurably affected the rate at which cells transition from the normal state R to the growth arrest state Q_a, we compared two different ways of modelling the clone-level vectors k_q. In the first model M1, k_q is treated like τ and k_d, i.e

$$\ln k_q = qconst + d_q * X + c_q$$
$$d_q \sim N(0, 0.3)$$

In the second design M2, we assume that there are no design-level effects, i.e.

$$\ln k_q = qconst + c_q$$

Clonal variation

We treated the clone-level parameters τ, q and k_d as determined by a common mean with either design and clone-level deviations or just clone-level deviations, depending on the parameter and the model iteration. In all cases the clone-level deviations have the following multi-level prior structure:

$$
\begin{aligned}
[c_\tau, c_q, c_d] &\sim multi\,normal(\mathbf{0},\ _{\mathbf{c}} \cdot \Omega) \\
\Omega &\sim lkj(2) \\
_{\mathbf{c}} \& sim log\,normal(-2.1, 0.35)
\end{aligned}
$$

Informative priors for non-design parameters

Other unkowns have informative prior distributions based on scientific knowledge:

$$
\begin{aligned}
\mu &\sim log\,normal() \\
R0 &\sim log\,normal() \\
qconst &\sim normal() \\
\tau const &\sim normal() \\
dconst &\sim normal() \\
d_\tau &\sim normal() \\
d_d &\sim normal()
\end{aligned}
$$

Data representation

We believed that there should be no noticeable effects due to the "bok" intervention, as this intervention knocks out a gene that is very weakly expressed in the control case. To verify that this was the case we compared the results of fitting the model M2 with and without distinguishing the "bok" design from the other designs.

Model comparison

To compare different models we calculated the approximate leave-one-timecourse-out log predictive density for models M1 and M2 on puromycin and sodium butyrate treatments using the python package arviz. See (Vehtari, Gelman, and Gabry 2017) for more about this model comparison method. For

timecourses where the pareto-k diagnostic was higher than 0.8, indicating that the approximation was inaccurate, we refitted the model in order to find the exact leave-one-out log predictive density.

We further evaluated our models using graphical posterior predictive checks and by inspecting the modelled parameter values.

For both treatments, the two models' estimated predictive performance was very similar.

Results

Was there a significant effect on k_q due to genetic interventions?

To assess whether the model with

Observed vs modelled timecourses

Figure 1. shows observed timecourses for each design under the 15ug/mL puromycin treatment, alongside a sample of model-realised timecourses. The modelled and observed timecourses appear qualitatively similar, suggesting that the model is not dramatically mis-specified.

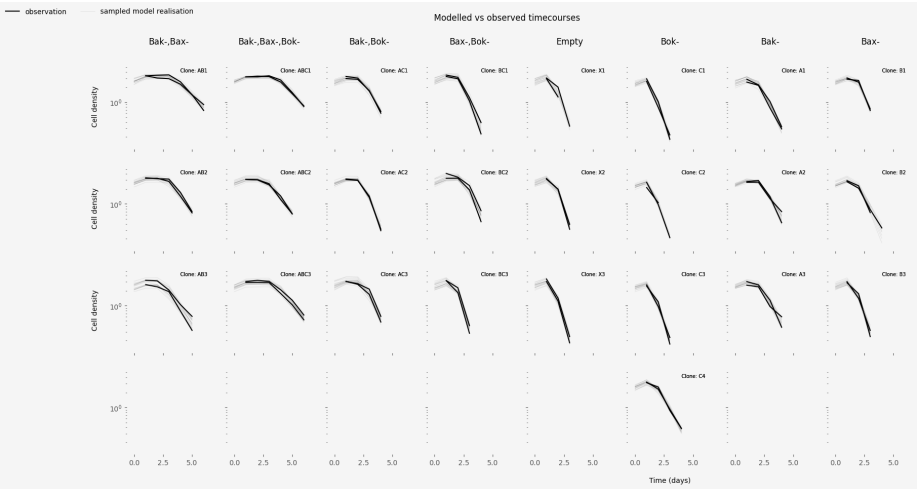

Figure 2: Simulated timecourses for the 15ug/mL Puromycin treatment

Posterior distributions of design parameters

Figure 2. plots the 2.5% to 97.5% marginal posterior intervals for the design-level parameters, relative to the control experiment. According to our model, some designs are probably different from the control with respect to the τ and k_d parameters. On the other hand, all of the posterior intervals for design-specific effects on the parameter k_q include zero, showing that the designs cannot conclusively be distinguished using the data provided.

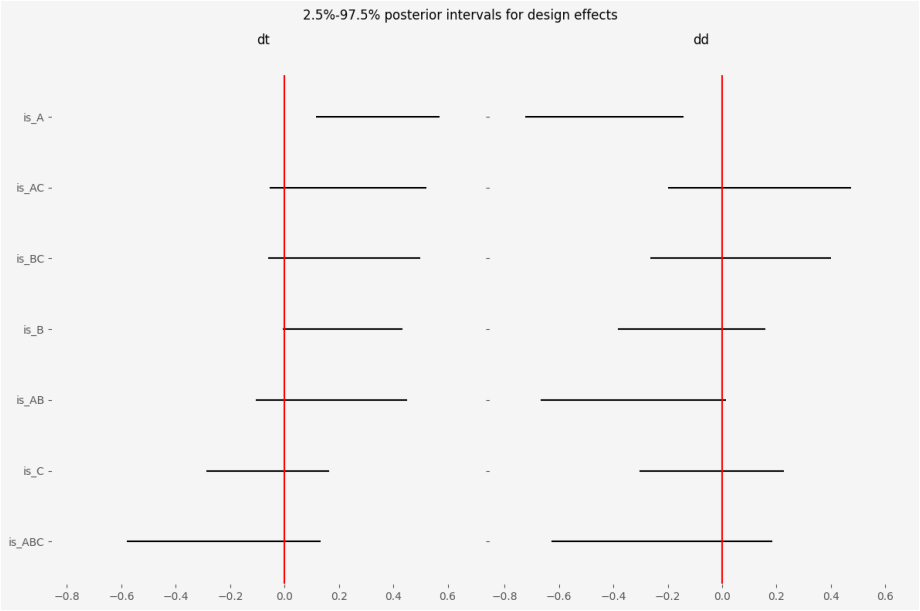

Figure 3: Posterior intervals for design level parameter

Vehtari, Aki, Andrew Gelman, and Jonah Gabry. 2017. "Practical Bayesian Model Evaluation Using Leave-One-Out Cross-Validation and WAIC." *Statistics and Computing* 27 (5): 1413–32. https://doi.org/10.1007/s11222-016-9696-4.